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Response of *Cognettia sphagnetorum* (Enchytraeidae) to manipulation of pH and nutrient status in coniferous forest soil

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With 3 figures

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1. Introduction

Enchytraeids are one of the dominating animal groups in terms of biomass in coniferous forest soil. In dry heath forests where earthworms are sparse or lacking, they may make up more than a quarter of total animal biomass (PERSSON *et al.* 1980). Because *Cognettia sphagnetorum* (VEJD.) alone makes more than 95 % of the total numbers of enchytraeids in this kind of site (NURMINEN 1967, LUNDKVIST 1982), it is probably the most important single species in the community which consists of hundreds of species.

Recent investigations focused on the role of soil animals in nutrient dynamics of the forest floor, indicate that in spite of their unimportant share in energy fluxes (PETERSEN & LUXTON 1982) their contribution to decomposition processes and nutrient mobilization may be of considerable importance (WITKAMP & AUSMUS 1976, REICHLER 1977, CROSSLEY 1977, McBRAYER 1977, ANDERSON *et al.* 1981).

Effects of fertilizer treatments on enchytraeids in coniferous forest soil have been treated in several studies (HUHTA *et al.* 1967, 1969, MARSHALL 1974, LOHM *et al.* 1977, ABRAHAMSEN & THOMPSON 1979). It is known that many fertilizers affect the soil acidity, which in turn will result in changes in microflora serving as nutrients for the soil fauna. The experimental design in these studies has not allowed separation of the role of pH from that of the nutrients added. More evidence has recently accumulated from experiments in which the influence of acidification and liming on the soil community has been tested (BÄÄTH *et al.* 1980, HÄGVAR & ABRAHAMSEN 1980). These have shown that manipulation of pH results in changes in the soil fauna.

The aim of the present study was to investigate the effects of different fertilizer treatments, including ash that has recently become an alternative to commercial products owing to its availability (waste product of energy production) and to the increasing prices of nitrogen fertilizers. Separate experiments were arranged in the field and laboratory in order to distinguish the effects of pH from those of nutrients.

This is part of a larger investigation. Other groups of soil animals, as well as microbiological parameters mainly focused on nitrogen fluxes, were also studied. Preliminary results of laboratory experiments (soil animals only) were published by HUHTA *et al.* 1983. Other results will be published later.

2. Material and methods

2.1. Field experiments and study sites

Study site 1 is located in Saarijärvi, central Finland (62° 40' N, 25° 15' E). It is a spruce stand of the *Myrtillus*-type, aged about 40 years at the beginning of survey. *Vaccinium myrtillus* dominates the field layer vegetation, which is rather sparse and patchy, leaving an almost continuous carpet of common forest mosses to cover the soil. The thickness of humus layer varies between 3 and 5 cm, locally up to 7 cm. The stand was thinned and the slash removed before the treatment. The soil of

Table 1. Nutrient analyses of untreated soil of the main study sites (Viljavuospalvelu Oy, Helsinki). The figures are the extreme values of three measurements, each based on a pooled sample from separate replicate plots.

		Saarijärvi		Tammela	
		Humus	Mineral	Humus	Mineral
Conductivity		1.6 — 1.9	0.5 — 0.6	1.2 — 1.4	0.3 — 0.4
pH		3.9 — 4.3	4.4 — 4.7	3.8 — 4.4	4.8 — 5.0
Ca exchangeable,	mg l ⁻¹	250 — 520	10 — 45	150 — 400	7 — 12
K exchangeable,	mg l ⁻¹	190 — 203	52 — 56	90 — 135	32 — 38
Mg exchangeable,	mg l ⁻¹	53 — 71	12 — 22	31 — 54	7 — 10
P easily soluble,	mg l ⁻¹	8 — 13	2 — 5	8 — 13	5 — 6
NH ₄ -N,	mg l ⁻¹	47 — 53	31 — 52	49 — 55	27 — 33
NO ₃ -N,	mg l ⁻¹	11 — 12 ¹⁾	1 — 25	7 — 9 ¹⁾	5 — 14

¹⁾ two measurements only.

Table 2. Chemical analyses of ashes

	Fly ash from Heinola timber factory (Enso- Gutzeit Oy)	Softwood bark ash used at site 3 (Viljavuospalvelu Oy)	Birch ash used for lab experiment (Viljavuospalvelu Oy)
Loss on ignition, %	20	22	n.d.
% soluble	48	n.d.	96.0
pH	11.1	12.7	12.7
P g kg ⁻¹	9	7	23
K g kg ⁻¹	38	15	98
Ca g kg ⁻¹	134	151	283
Mg g kg ⁻¹	15	11	40
S g kg ⁻¹	15	3	n.d.
Fe g kg ⁻¹	12	n.d.	n.d.
Na g kg ⁻¹	5	n.d.	n.d.
Mn g kg ⁻¹	8	n.d.	n.d.

Note: n.d. = not determined.

the control plots was analysed for contents of main nutrients in November 1979. The results are shown in Table 1.

This site was designed for studying the effects of different nitrogenous fertilizers applied in quantities normally used in forestry. 40 × 40 m test plots were set up in an area of ca. 2 ha. These were randomized for four different treatments, two replicates for each, i.e.

C: Control, unfertilized.

U: Urea (H₂NCONH₂), 432 kg ha⁻¹, i.e. 200 kg N ha⁻¹.

AN: Calcium ammonium nitrate ("Oulu-saltpetre", Kemira Oy), 727 kg ha⁻¹, i.e. 200 kg N ha⁻¹. (Later referred to as ammonium nitrate).

U + PK: Urea plus phosphorus and potassium in form of apatite (200 kg ha⁻¹) and biotite (1560 kg ha⁻¹), i.e. ca. 100 kg P₂O₅ ≈ 44 kg P and 100 kg K₂O ha⁻¹ ≈ 83 kg K.

The fertilizers were spread at the end of May 1979. In each main plot, a subplot of 10 × 10 m was set up for soil zoological studies. One duplicate of the U + PK treatment was later considered unrepresentative on the basis of botanical survey, and both subplots were taken from opposite sides of the same main plot.

Site 2 is located in Tammela, southern Finland (60° 40' N, 23° 50' E). It is a pine stand of the *Calluna*-type, ca. 50 years at the start of survey. *Calluna vulgaris* dominates the field vegetation, otherwise it is similar to site 1. The results of nutrient analyses are shown in Table 1.

The experimental design was the same as in study site 1. In addition, smaller plots (10 × 10 m) were established for fertilization with ash. One plot (symbol A) was treated with 7,000 kg ha⁻¹ fly ash from a power plant of a timber factory. The second plot (symbol A + P) received the same amount of ash added with apatite (100 kg P₂O₅ ≈ 44 kg P ha⁻¹). The addition was done because ash alone is poor in phosphorus (Table 2). The ash available for the experiment was of low quality and therefore applied in rather a large amount. The treatment was done in May 1979. Subplots of untreated main plots served as controls. In 1982 an extra control plot close to plots A and A + P was also sampled.

Site 3 is a young (ca. 30 years) *Calluna*-type pine stand at Ruotsinkylä near Helsinki (60° 25' N, 24° 50' E). It was thinned in the previous year. *Calluna vulgaris* strongly dominates the field vegetation.

Ten 4 × 4 m test plots were selected from as homogeneous places as possible, most of them with a young pine in the centre. Slash, if present, was removed from the plots. Each plot was further divided into four subplots, and these were randomized for different treatments.

Fertilizers were spread on the plots at a 19 days' interval (13 May and 1 June 1981; the second treatment was not done before there had been rain). The treatments were:

C: Control, unfertilized.

U: Urea, $2 \times 230 \text{ kg N ha}^{-1}$.

A + P: $2 \times 3.350 \text{ kg ha}^{-1}$ (dry mass) bark ash + $2 \times 500 \text{ kg ha}^{-1}$ commercial superphosphate equivalent to $200 \text{ kg P}_2\text{O}_5 \approx 88 \text{ kg P ha}^{-1}$ (total).

L: Slaked lime, Ca(OH)_2 ("Fine Lime", Lohja Oy), $2 \times 2.000 \text{ kg ha}^{-1}$.

This experiment was planned to study the short-term effects of pH and nutrients, and to check the results from the main field experiments (sites 1 and 2). Double doses of nutrients were used in order to demonstrate possible changes with higher reliability. Superphosphate was chosen instead of slowly soluble apatite, and calcium hydroxide instead of crushed limestone, in order to observe the effects more rapidly. The amount of lime was calculated to result in a similar change in soil pH as did the ash-treatment. This was based on laboratory experiments, where different amounts of ash and lime were spread on the surface of undisturbed soil samples. These were watered at times, and the pH of humus was measured repeatedly over two months.

2.2. Laboratory experiments

Laboratory tests were designed to separate the effects of pH from those caused by addition of nutrients. Because in undisturbed soil it was not possible to produce similar vertical pH gradients with ash and lime, the materials were mixed homogeneously with soil. A preliminary test was first made in order to find out what amounts of ash or lime were needed to produce a desired rise of pH.

Intact blocks of soil (all the organic layer including vegetation) were taken from study site 3 (see above), and placed into six plastic boxes measuring 40 × 60 cm, 11 cm depth. Before insertion, ca. 1 cm of mineral soil was taken from immediately under each plot and spread on the bottom of the box. Organic soil from the same site was brought into the laboratory and sieved through a 10 mm mesh, forcing by hand most of the dead organic matter through the sieve. The soil so treated was mixed and divided for different treatments. The minerals to be tested were weighed and mixed thoroughly with this soil.

Equal portions of these test materials (soil with or without minerals) were weighed and placed into small baskets made of plastic mesh (\varnothing 4 cm, height 8 cm, mesh 1.5 mm). When loose at the beginning, the samples made about 100 cm³ in volume. Holes of corresponding size were bored into the soil in the boxes. Each box had 54 holes and received 18 replicates of three test materials. The order of the samples was random-systematical: each row of six holes received two replicates of each test material, the position of which in the row was randomized. The boxes were covered with perforated plastic to reduce evaporation, and incubated in clima chambers in 12 + 12 h daily cycles of +20 and +15 °C (light off at night). These temperatures correspond to field temperatures recorded from humus of a clear-cut area during a warm period in August 1972 (HUHTA & MIKKONEN 1982).

The soil in the boxes was kept moderately moist by watering at times with distilled water. Winter conditions were simulated during the experiments by lowering the temperature weekly by steps of 5 °C, until it was close to zero under 24 hour dark conditions for 2 or 3 weeks. Summer conditions were then re-established by a reverse procedure. The order of the boxes on the shelves of the two chambers was changed weekly.

Experiment 1 was started on 13 August 1981 and kept till 18 May 1982 ("winter" between 1 Dec. and 2 Febr., lowest temperature $+3 \pm 1$ °C). The test materials were:

C: Control, mixed soil with no addition.

A: 9.7 g (d.m.) birch ashes and 1.4 g superphosphate kg⁻¹ (f.m.) of soil, equivalent to 1.750 kg ashes and 50 kg P₂O₅ $\approx 22 \text{ kg P ha}^{-1}$.

L: Slaked lime, Ca(OH)_2 , the same amount as ashes in A.

Both ash and lime were sieved before weighing through 0.57 mm mesh. Superphosphate was pulverized in a mortar. The portions of test materials weighed 33 g (fresh mass), corresponding to 18.2 cm² *in situ*.

Experiment 2 was started on 25 May and kept until 7 December 1982 ("winter" between 10 Sept. and 5 Nov., lowest temperature -2 ± 1 °C in chamber 1, and 0 ± 1 °C in chamber 2). The test materials were:

C: Control.

U: Urea, 2.6 g kg⁻¹ of soil (fresh mass; water content 56%), equivalent to 150 kg N ha⁻¹.

AN: Ammonium nitrate, 3.4 g kg⁻¹, which makes the same amount of nitrogen as in U.

The chemicals were in pulverized form for analytical purposes. The weighed samples (25.1 g f.m.) correspond to 20 cm² *in situ*.

In addition, 100 g portions of the same test materials used for Experiment 2 were weighed into separate plastic vessels. These were used to follow the development of populations in conditions when invasion to and from the surrounding soil was prevented.

2.3. Sampling and sample treatment

In each field plot at study sites 1 and 2, 5 permanent points were marked (ten on plots A and A + P at Tammela) on homogeneous places. Succeeding samples were taken as close to these points as possible, yet from undisturbed places and at a minimum distance of 10 cm from previous samples. This procedure was thought to minimize accidental variation between sampling dates in comparison with strictly random sampling.

At study site 3, samples were taken from random points of each subplot. Thus a total of 10 units was taken each time from each treatment (15 including the extra control plot in Tammela 1982).

The samples, 25 cm² in area were taken with a cylindrical steel corer, inside which plastic rings were inserted. The cores were cut between the rings into 3 cm layers and transported separately in plastic bags into the laboratory. Animals were extracted with the wet funnel technique of O'CONNOR (1962). The longest storage in a refrigerator before extraction was 10 days.

For pH measurements, 5 similar units from each plot were taken with a 10 cm² corer, pooled and mixed well. A subsample of ca. 5 g was mixed into 60 ml distilled water, and pH was recorded after 1 hour's incubation (overnight for 1980 samples from Saarijärvi).

From the laboratory experiments, one unit of each treatment (all from the same randomly chosen row), together with its mesh-basket, was removed at selected intervals from each of the six boxes, and extracted immediately. From the separate vessels, samples of about 75 cm³ in volume were taken with a plastic ring. pH was measured from two separate samples, pooled from 3 units each. In the experiment 2 conductivity was measured from the same samples.

The exact sampling dates were as follows:

Saarijärvi: 28 May, 26 June, 23 July, 20 Aug. and 18 Sept. 1979, 16 May, 16 June, 14 July, 11 Aug. and 8 Sept. 1980, 26 May, 8 July and 15 Sept. 1981, 1 June, 18 July and 13 Sept. 1982.

Tammela: 10 May, 9 June, 14 July, 11 Aug. and 13 Sept. 1980, 14 May and 16 June 1981, 1 June, 19 July and 14 Sept. 1982.

Ruotsinkylä: 9 July, 30 July, 15 Sept. 1981, 21 May, 5 July and 27 Aug. 1982.

The sampling dates of the laboratory experiments can be seen in Table 4 and Fig. 3.

After extraction the animals were stored in alcohol, and later identified and counted under a binocular microscope. This was done by technical staff not experienced in taxonomy of enchytraeids. It is known from the study of NURMINEN (1967) that only 4 enchytraeid species occur regularly in coniferous forest soils in southern and central Finland: *Cognettia sphagnetorum* (VEJD.), *C. glandulosa* (MICH.), *Mesenchytraeus flavus* (LEV.) and *Bryodrilus ehlersi* UDE. *C. sphagnetorum* usually makes up 98 to 100 % of total numbers. LUNDKVIST (1982) also reported a 99 % dominance of *C. sphagnetorum* in a pine stand at about the same latitude, all other specimens found belonging to *Mesenchytraeus* spp. *Mesenchytraeus* and *Bryodrilus* are easy to identify by their external appearance. *C. glandulosa* can not be distinguished by an untrained person, but it occurs in very low numbers. All other species are rare. So the technicians were trained to differentiate between "thick" (*M. flavus* and *B. ehlersi*) and "thin" (*Cognettia* spp.) specimens; the latter thus include all other species, but misidentifications are so few that the numbers so obtained can be regarded to represent *C. sphagnetorum* only. The two "thick" species occurred only sporadically in the samples.

Using a 2 mm scale in the bottom of counting vial, the animals were measured into size classes (1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 15 mm). 110 preserved specimens were measured individually (thickness at 3 points, 1/4, middle and 3/4) for calculating body mass (ABRAHAMSEN 1973), after which a length-mass regression was counted ($M = 17.0 \cdot L^{1.45}$; $r = 0.90$). Using a dry mass-wet mass ratio 0.16 (PERSSON *et al.* 1980) average dry masses were obtained for each size class for the estimation of population biomass.

Differences in population densities between treatments were tested with the aid of two-way analysis of variance after logarithmic transformation of the data. This was done separately for each year and each pair of treatment. The data from the laboratory experiments were tested with the student's *t*-test without transformation, separately for each sample.

3. Results

3.1. Nitrogen fertilizers

All nitrogen fertilizers applied in a quantity recommended in practical forestry (Saarijärvi, Fig. 1) reduced the numbers of *C. sphagnetorum* by about 50 % (mean of the first summer). The difference was greatest in the first samples after the treatments, after which it remained at a constant level through the second year. There was no difference between the three

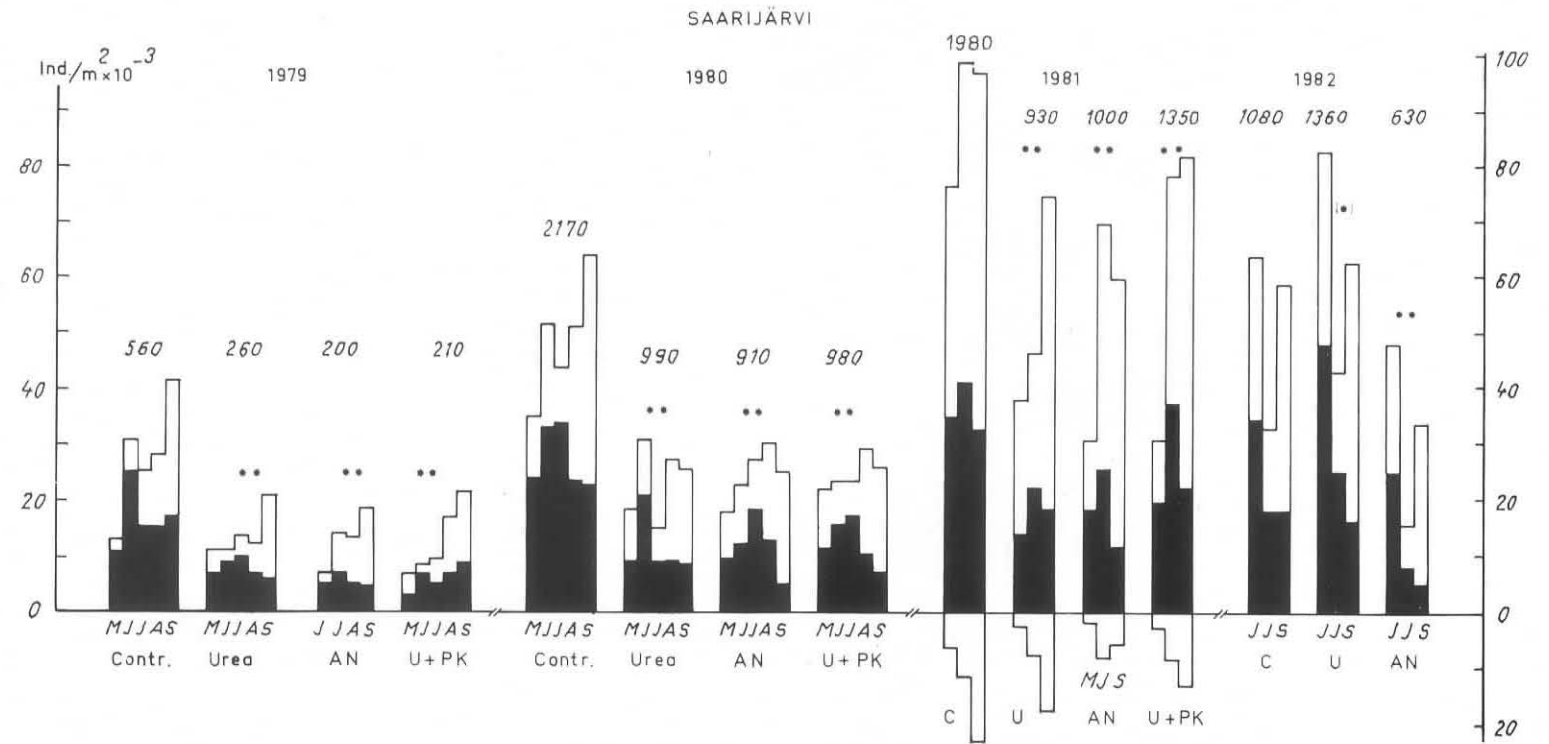


Fig. 1. Numbers of *C. sphagnetorum* during four years after different treatments at study site 1 (Saarijärvi). The white parts of histograms refer to the 0—3 cm horizon, and the black parts to 3—6 cm. The white parts below base line in 1981 refer to 6—9 cm. Annual mean biomasses in mg dry masses m^{-2} are given above the graphs. Asterisks indicate significant differences in total numbers (0—6 cm) from the control at probability levels $P < 0.05$ (*) and $P < 0.01$ (**) (in parentheses when the difference was significant in one duplicate only). C = control, U = urea, AN = ammonium nitrate, PK = phosphorus and potassium.

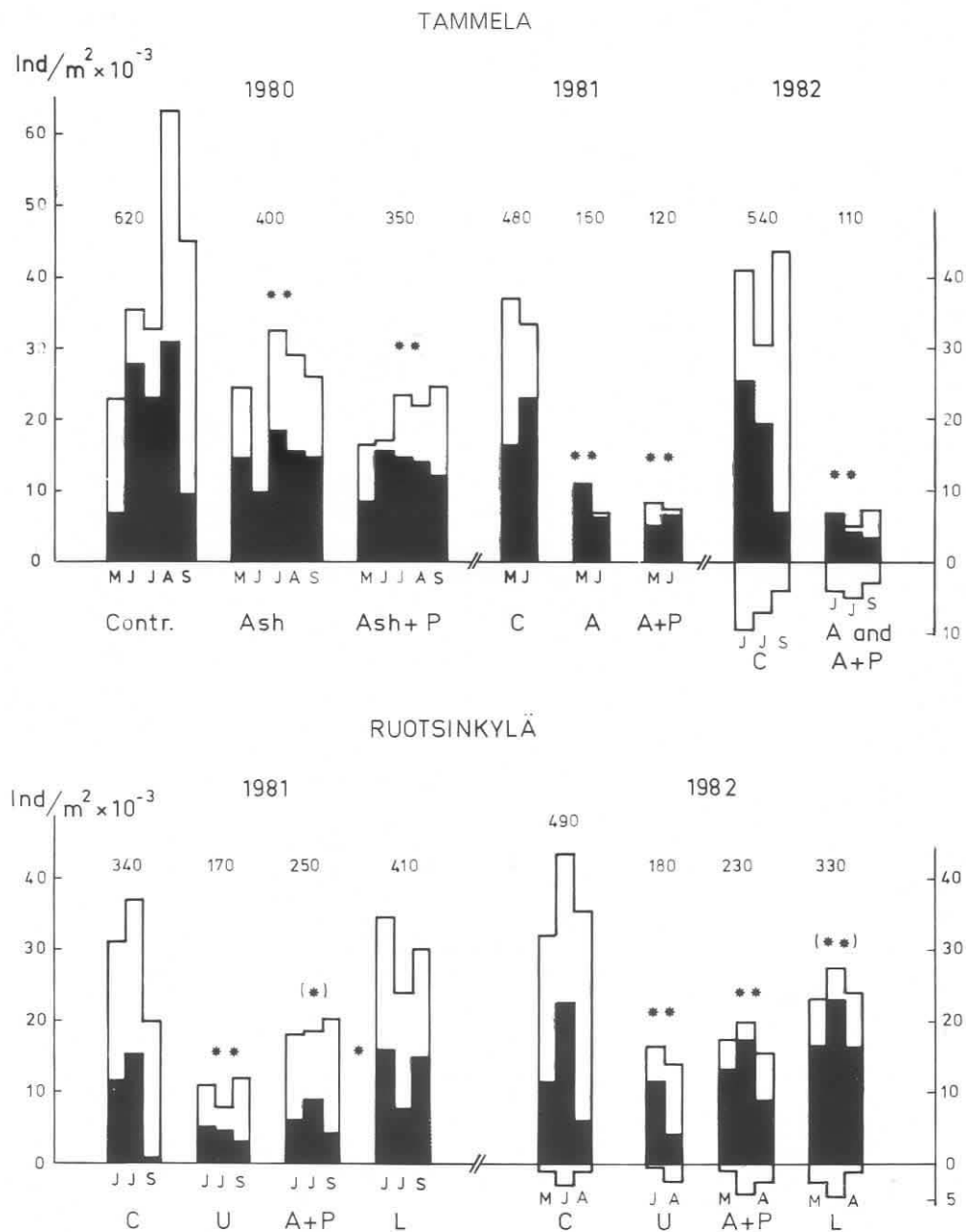


Fig. 2. Numbers of *C. sphagnetorum* after different treatments at study sites 2 (Tammela) and 3 (Ruotsinkylä). The asterisk between two graphs indicates difference between respective treatments, and those in parentheses refer to cases when the difference was significant in one soil layer only. C = control, A = ash, A + P = ash + phosphorus, U = urea, L = lime. Other explanations as in Fig. 1.

Table 3. Difference (%) from the control in annual mean numbers of *C. sphagnetorum* after different treatments

	Layer, cm	Year after treatment			
		1st	2nd	3rd	4th
Saarijärvi					
Urea	0—3	—47	—44	—36	+22
	3—6	—53	—58	—50	+25
	0—6	—54	—52	—41	+23
	6—9	n.d.	n.d.	—29	n.d.
NH ₄ NO ₃	0—3	—43	—39	—36	—31
	3—6	—73	—58	—49	—48
	0—6	—58	—49	—41	—38
	6—9	n.d.	n.d.	—61	n.d.
Urea + PK	0—3	—41	—42	—36	
	3—6	—62	—54	—28	
	0—6	—55	—49	—32	
	6—9	n.d.	n.d.	—40	
Tammela					
Ash, ash + P	0—3		—56	—89	—90
	3—6		—30	—67	—73
	0—6		—44	—78	—83
	6—9		n.d.	n.d.	—43
Ruotsinkylä					
Urea	0—3	—69	—69		
	3—6	—55	—43		
	0—6	—65	—59		
	6—9	n.d.	— 6		
Ash + P	0—3	—39	—81		
	3—6	—24	— 2		
	0—6	—35	—53		
	6—9	n.d.	+52		
Lime	0—3	— 8	—74		
	3—6	+30	+39		
	0—6	+ 3	—33		
	6—9	n.d.	+64		

treatments. The populations seemed to be equally affected in both horizons (0—3 and 3—6 cm), the average numbers being roughly the same in both, and showing parallel seasonal variation.

Towards the third autumn the populations started to show some recovery in the plots treated with urea (with or without P and K). This trend continued so that in the fourth summer the numbers already exceeded the control level, although the difference was significant in one replicate only. (The two replicates of urea treatment differed from each other throughout the study period, but pooled data have been presented in Fig. 1 and used in statistical tests). The plots treated with ammonium nitrate showed virtually no recovery even during the fourth year.

Only a minor part, 10—15% of the population was found in 6—9 cm (1981). As far as can be concluded from three samples the fertilization seems to have affected this layer as strongly as the organic horizon, at least by the third summer.

A double dose of urea (Ruotsinkylä, Fig. 2) caused an even more marked decrease in the population, with no recovery by the second autumn. As an average, the density remained 62% lower in comparison to the control. In this case the effect was stronger in the topmost soil layer than in 3—6 cm (Table 3). In the mineral soil (6—9 cm) the numbers of worms were negligible.

In the laboratory experiment (Fig. 3) both nitrogen compounds reduced numbers of *C. sphagnetorum* to a fraction of those found in untreated soil. Ammonium nitrate proved to be acutely toxic, destroying virtually the whole population during the first two days. Urea caused only an insignificant reduction at first, but then the numbers decreased continuously. Relative numbers were lowest two months after the treatment (—95%). Later the population gradually recovered, but because of simultaneous increase in the control soil, the density was only 28% of the control even at the last sampling. A corresponding recovery took place after the treatment with ammonium nitrate. Contrary to the urea treatment, the numbers finally (last samples) even exceeded the control level.

The duplicate experiment in separate boxes revealed that the recovery of the population was largely attributable to invasion of animals from the surrounding untreated soil. On 7 September, when the numbers were 20% of the control in the urea treatment and 24% in AN in the main experiment, they were 0 and 1%, respectively, in separate vessels.

3.2. Non-nitrogen fertilizers

Fertilization with ashes, with or without addition of phosphorus, caused a strong and long-lasting decline in the population. Although there were some deviations in individual samples (which may be explained by high variation), the average trend is clear: the numbers decreased from year to year up to the fourth growing season after the treatment. While the mean population density in Tammela was 39% (A) to 48% (A + P) below the control level in 1980 (second year), the difference was about 78% in 1981 and 83% in 1982 (Table 3). Correspondingly, the data from Ruotsinkylä show a reduction by 35% in the first summer (1981), and 53% in the second.

The response was clearly more marked in the topmost 3 cm than deeper in the soil (Table 3). When the population density in 0–3 cm was reduced up to 90% (1982), the reduction was only 43% at the same time in 6–9 cm. The same can be seen even more clearly in the data from Ruotsinkylä: In the second year (1980) the mean population density was decreased by 81% in 0–3 cm. In 3–6 cm there was almost no difference from the control, and in the mineral soil the numbers were even higher by 52%. However, because only an insignificant part of the population lived in 6–9 cm, this had little influence on the total picture (Fig. 2).

Treatment with ashes and phosphorus showed an equally detrimental effect in the laboratory experiment (Fig. 3). There was a strong increase of worms in the control soil until March 1982, after which they decreased again. This is in good accordance with the results by ABRAHAMSEN (1971). He observed maximum abundance after ca. 6 months' incubation at +18 °C, when no fresh material was added into the culture (homogenized raw humus). In comparison with the control, the population in ash-treated soil was lowest (—90%) about half a year after treatment. 3 months later the difference had reduced to 50%. The fertilizer was not acutely harmful, because 1 to 2 days after mixing of the materials the numbers were only insignificantly lower than those in untreated soil (test for showing the immediate effect was arranged in connexion with the second experiment, see Fig. 3).

3.3. The role of pH

The role of pH in the effects of fertilizers can be examined best in the laboratory experiments that were especially planned for this purpose (Fig. 3). In experiment 1 the soil pH

Fig. 3. Numbers of *C. sphagnetorum* per sample unit (ca. 100 cm³) in the laboratory experiments. C = control, A = ash + phosphorus, L = lime, U = urea, N = ammonium nitrate, S = salt. Standard errors are shown as vertical lines. Significant differences from the controls at probability levels $P < 0.05$ (*) and $P < 0.01$ (**) are given above respective columns. Differences between two treatments are shown under the columns. Experiment 1 was started on 13 August, and Exp. 2 on 25 May.

Table 4. Results of pH measurements

Laboratory experiments¹⁾

Exp. 1	30 Aug.	5 Oct.	1 Dec.	9 March	18 May		
Control	4.5	4.4	5.0	5.0	4.8		
Ashes	6.9	6.7	6.6	6.3	5.4		Separate
Lime	7.0	6.6	6.6	6.5	5.5		boxes
Exp. 2	25 May	2 June	15 June	5 July	27 July	7 Sept.	8 Sept.
Control	4.4	4.3	4.3	4.7	4.7	4.9	4.9
Urea	6.5	6.4	5.8	6.0	5.9	6.1	6.0
AN	3.8	4.1	4.1	4.9	4.9	5.0	4.6

Ruotsinkylä

Symbol	Layer	1981		1982		1983
		June	Aug.	May	July	May
C	0—3	4.5	4.0	4.3	—	4.7
	3—6	4.4	4.3	4.5 ²⁾	4.7	4.7
	6—9	—	—	4.7 ²⁾	4.7	4.9
U	0—3	—	6.0	5.7	5.2	4.9
	3—6	—	5.1	5.0 ²⁾	5.2	4.7
	6—9	—	—	5.2 ²⁾	4.9	4.9
A + P	0—3	7.4	6.5	6.7	7.0	7.2
	3—6	5.7	4.5	4.6 ²⁾	6.0	4.7
	6—9	—	—	4.7 ²⁾	4.9	5.0
L	0—3	7.4	6.6	7.3	7.0	7.7
	3—6	4.6	4.4	4.8 ²⁾	6.2	4.9
	6—9	—	—	4.7 ²⁾	5.4	5.0

Tammela

Symbol	Layer	1979 ¹⁾		1980 ¹⁾		1982
		June	Sept.	June	July	Sept.
C	0—3	4.1	4.0	3.7	4.3	4.3
	3—6	—	—	—	4.3	4.1
	6—9	—	—	—	4.5	4.2
A ²⁾	0—3	4.8	5.6	6.0	6.0	6.0
	3—6	—	—	—	5.1	5.5
	6—9	—	—	—	5.2	4.8
A + P	0—3	4.8	6.3	5.9	5.2	5.8
	3—6	—	—	—	4.7	5.5
	6—9	—	—	—	4.6	5.0

Saarijärvi

Symbol	Layer	1980						1982			
		May	June	July	Aug.	Sept.	mean	June	July	Sept.	mean
C	0—3	4.3	5.1	4.1	4.2	3.8	4.3	4.4	4.7	4.2	4.4
	0—6	4.0	5.1	3.9	4.1	4.0	4.2	4.3	4.4	4.3	4.3
U	0—3	5.9	6.2	5.5	5.1	4.6	5.5	4.5	4.8	4.3	4.5
	3—6	4.8	5.4	4.3	4.9	4.2	4.7	4.4	4.6	4.3	4.4
AN	0—3	4.5	5.3	4.2	4.5	4.1	4.5	4.5	4.8	4.3	4.5
	3—6	4.1	4.0	4.0	4.2	3.9	4.2	4.4	4.6	4.3	4.4
U + PK	0—3	5.6	5.8	5.0	4.6	4.5	5.1	—	—	—	—
	3—6	4.7	5.3	4.2	4.2	4.1	4.5	—	—	—	—

Note: Each number is a mean of two measurements made from separate pooled samples.

¹⁾ Measures from total organic horizon.

²⁾ One measurement only.

was manipulated with calcium hydroxide to the same level as after ash-treatment. As can be seen from Table 4, the operation was rather successful; the pH of the two treatments differed at most by 0.2 units at any sampling time. In two of four samples the numbers of *C. sphagnetorum* were identical in the two treatments. In two others they differed in opposite directions, and significantly in one sample only. On the basis of this experiment it seems that change in pH alone can explain the decrease of population, leaving little importance to the nutrients applied.

The experiment at Ruotsinkylä (Fig. 2) was designed for testing the role of pH in field conditions. In the first year the result seemed contradictory with that of the laboratory test: the population did decrease in ash-treated plots, but did not in the limed ones, in spite of the fact that the pH was almost the same in both (with the exception of 3–6 cm layer in June when no samples were taken). However, in the next year both treatments were clearly more similar to each other than to the control plots. The pH was the same or somewhat higher in the limed plots than in the ash-treated ones. The observed results may be explained by lower solubility of $\text{Ca}(\text{OH})_2$ than at least some components of ash. In fact, lime could be seen on soil surface even in the second summer, partly in the form of granules. Ca^{++} ions are also more easily absorbed by soil particles than are K^+ and Na^+ ions (TROEDSSON & NYKVIST 1973). Ash-treatment caused a rapid temporary increase of pH in the 3–6 cm soil depth, while the long-lasting decrease of acidity in the deeper horizons took place only slowly. Thus the field experiment finally supports the observations from the laboratory test. However, the bulk pH does not necessarily reveal the situation in the micro-environments in the soil.

The laboratory experiment 2 was designed to compare two nitrogen compounds, one of which is acid and the other (urea) results in a rise of soil pH. The results remain somewhat obscure, firstly because of the acute toxicity of ammonium nitrate. So the pH had no role in the destruction of the population after this treatment. Attention should be focused on the later samples when toxicity can be expected to disappear owing to microbial immobilization and transformation of nitrogen, while difference in pH persisted (Table 4). If pH is the principal regulator, the population in the NH_4NO_3 treatment should approach that in untreated soil. On the other hand, if a decisive role is played by nitrogen, the situation should remain similar with urea treatment, because the same amount of N was present in both.

Numbers of worms increased gradually in both treatments, but they did also in the control soil (Fig. 3). From late July onwards, mean numbers were somewhat higher in the ammonium nitrate than in the urea treatment, but in September the difference was insignificant. Up to this date the observations indicated that the populations were regulated by the amount of nitrogen. However, after experimental winter conditions the population had increased explosively in the NH_4NO_3 treatment, but remained unaltered in the urea treatment. This can be regarded as evidence for the decisive role of pH. It remains uncertain whether the population was still suppressed by toxicity of ammonium nitrate in September.

Considering the decisive role of pH in the influence of non-nitrogen fertilizers, the long-lasting decrease of the population after ash-treatment is easily understood: in the fourth year the pH of treated plots still differed by 0.9 to 1.7 units from that of control plots in the topmost soil layer, 0.4 to 1.4 units in 3–6 cm and 0.1 to 0.8 units in 6–9 cm (Table 4). This corresponds well with the distribution of the population between these layers (Fig. 2).

It is also obvious from Table 4 that the neutralizing effect of urea is of long duration. At normal dosages used in Saarijärvi, there was a continuing average difference of about one pH unit between fertilized soil and control still through the second summer. This could explain the continued depression of the enchytraeid population. The difference of acidity had disappeared by the fourth summer, which is also in agreement with the recovery of the population. However, it is in contradiction with the permanent depression of numbers in plots treated with ammonium nitrate, in which the pH did not essentially differ from the control at any time.

4. Discussion

The negative response of *C. sphagnetorum* to nitrogen fertilizers, as well as the later recovery of the population, has also been documented in previous investigations. After application of a multiple fertilizer, including 90 kg N ha⁻¹ in NH₄NO₃, HUHTA *et al.* (1967, 1969) observed a steep decline of Enchytraeidae followed by a recovery up to twice above the control level by the third growing season. Both reactions were stronger than in the present study, taking into account the smaller amount of nitrogen applied. The role of other nutrients that were also in easily soluble form, could not be separated in their study. MARSHALL (1974) reported a decrease of Enchytraeidae (unidentified) after urea fertilization (224 and 448 kg N ha⁻¹) in a Douglas fir stand. The population recovered close to the original level during one year, after which the survey was not continued. LOHM *et al.* (1977), who made several experiments in Scots pine forests, did not observe significant response in *C. sphagnetorum* after application of 150 kg N ha⁻¹, either as urea or ammonium nitrate, and given in single or repeated applications. Instead, when increasing the dosage to 480 kg N ha⁻¹ (three applications of NH₄NO₃) the population decreased to 17% of the control. ABRAHAMSEN & THOMPSON (1979) applied urea in a mixed coniferous stand in three dosages. 100 kg N ha⁻¹ had no harmful effect, but a significant increase of *C. sphagnetorum* took place in the third year. 400 kg N ha⁻¹ affected negatively at first and positively after two years. 1600 kg N ha⁻¹ virtually destroyed the population, but after several years an even more marked increase took place.

Thus the available data are in rather good agreement, but there are differences in the strength of reaction or dosage needed to cause a significant response. Experiments are lacking about the influence of nutrients other than nitrogen. The role of pH in the context of fertilizing has been discussed by LOHM *et al.* (1977) and ABRAHAMSEN & THOMPSON (1979), but mainly with regard to short-term effects of urea application. Urea is hydrolysed in soil resulting in NH₄⁺ ions, some of which are transformed into volatile ammonia especially in alkaline conditions. The rise of pH during hydrolysis thus favours volatilization, and gaseous ammonia is very toxic to soil animals (MOURS 1962). In the present laboratory experiment there was no significant decrease of population within 2 days after mixing of the chemical into soil (Fig. 3), and hydrolysis of urea is completed in a few days (e.g. OVERREIN 1968). So the assumption of acute toxicity does not hold for the dosage of urea used, as was also considered by ABRAHAMSEN & THOMPSON (1979). On the contrary, ammonium nitrate that temporarily even lowered the pH, was toxic enough to decimate the population.

Another hypothesis is the "salt effect": when the fertilizer is dissolved into soil water, its osmotic potential may increase to such an extent that tolerance of soft-skinned animals is exceeded. To test this, NaCl was mixed into a portion of experimental soil in an equivalent amount to result in the same osmotic potential as does NH₄NO₃ when completely dissolved. Two days after treatment the conductivity was almost identical in salt-treatment and AN-treatment: 330 and 355 μ S cm⁻¹, respectively; 80 in urea, 100 in ash and 10 in the control. (In later samples, conductivity remained higher in AN than in U, but by the end of July, it had reduced to ca. 120 in AN and 75 in U. Higher values were sometimes recorded from the control soil). Salt did decrease the population significantly, but clearly less than did ammonium nitrate (Fig. 3). This indicates direct toxicity of ammonium nitrate itself. There was no "salt effect" after the application of urea or ash. HEUNGENS (1980) reported a negative correlation between *C. sphagnetorum* and conductivity in NPK-fertilized pine litter, but this was after 1 to 3 months' incubation and the result was not necessarily due to osmotic potential.

It is possible that both osmotic potential and small amounts of un-ionized ammonia may reduce survival and reproduction even if not enough to cause direct mortality. However, in the long run, the influences of the treatments are more likely explained by long-term changes in soil properties, namely by pH (if altered) and/or increased concentration of nutrients. It has been shown by BÅÅTH *et al.* (1980) that liming (but also acidification) dramatically reduces population of *C. sphagnetorum*, and by HÅGVAR & ABRAHAMSEN (1980), that increased

pH slows down colonization of sterilized soil samples by the same species. Thus mere manipulation of pH has been proved to affect the enchytraeids even without addition of nutrients (calcium as an element is not considered to be a limiting factor).

Soil acidity seems to be a principal factor among the environmental requirements of *C. sphagnetorum*. STANDEN & LATTER (1977) found indications of an optimum pH between 3.6 and 3.8, and STANDEN (1982) observed a significant negative correlation between pH and population density of this species in British grasslands. HEALY (1980) showed in an extensive field survey that soils with an abundant population of *C. sphagnetorum* had pH between 3.0 and 5.5 (highest numbers between 3.0 and 4.5), while the species occurred commonly within a wide range of soil moisture. Treatment with urea or ash clearly raises the pH above this optimum range (Table 4).

With respect to non-nitrogen fertilizing, the population density of *C. sphagnetorum* correlates so well with pH that no other factor seems necessary for explaining the effects of the treatments. In the case of nitrogen fertilizers, changes in pH offer only a partial explanation. Some further factors seem to be involved, possibly in interaction with acidity: Why do the numbers increase above control level after some period of depressed populations after urea treatment? Why did ammonium nitrate result in a similar decrease in spite of unaltered acidity? And, why was there no later increase corresponding to that after urea fertilization? Also, it should be noted that the difference in population density was somewhat greater between urea and control than between ash and control, although the difference in pH was smaller in the former case (Fig. 3).

It is rather unlikely that either pH or nutrient concentration would affect the enchytraeids directly. Probably the influence comes indirectly through changes in microbial populations, possibly also in the quality of soil organic matter, and in later phases in the quality and quantity of litter. Unfortunately, the nutritional requirements of *C. sphagnetorum* are incompletely understood. LATTER (1977), SPRINGETT & LATTER (1977) and LATTER & HOWSON (1978) showed that specimens taken from field populations in blanket bogs feed on dead litter of *Rubus*, *Eriophorum* and *Calluna*. They can be grown in pure cultures of fungi while presence of bacteria may actually kill the animals. When fed with *Rubus* litter, animals grew well if the litter had previously been bleached by a certain fungus, but died in unbleached litter and in cultures of the same fungus. It was concluded that *C. sphagnetorum* derives its main nutrition from litter preconditioned by microbes, and that the species can be regarded as a primary decomposer of detritus. There was no indication that bacteria would be used as an essential food source, and even fungal hyphae were reported to pass unaltered through the digestive tract. DASH & CRAGG (1972) and ANDERSON (1975), however, have shown that fungi are selectively ingested and also digested at least by some species other than *C. sphagnetorum*.

This knowledge leads us a little forwards in understanding the reaction of *C. sphagnetorum* to fertilizer treatments. According to FRANZ (1959) soil bacteria increase strongly after liming, while fungi are depressed. A review by MAYER-KRAPOLL (1963) revealed that stimulation of bacteria after liming has been reported by several authors, while the situation is more variable concerning fungi. An increase of bacteria was also observed in our fertilized plots in Tammela (UOMALA, unpublished) but fungi were not measured. SCHALIN (1967) has shown in a Finnish pine forest that urea fertilization even in a quantity of 100 kg N ha⁻¹ results in a rapid and strong increase of bacteria, while the number of microfungi decreases coincidentally (plate count method). Calcium ammonium nitrate and nitrate of lime caused an increase of both microbial groups, but of much smaller magnitude. Ammonium sulphate showed quite an opposite effect, decreasing the numbers of bacteria, and increasing fungi. It was considered that soil pH that was differently affected by these fertilizers was the principal factor to explain the observed phenomena. Both groups of microbes reacted positively to fertilization when the pH remained close to 4.3. Above this level there was an increase in bacteria and decrease of fungi, and below pH 4.3. the opposite occurred. A positive correlation between pH and bacteria was also observed at our study site in Tammela (UOMALA, unpublished).

In the experiments of BÄÄTH *et al.* (1980) bacterial biomass decreased and total biomass of fungi increased with lowering of pH, in spite of a decrease of active fungal hyphae. Liming, however, did not affect the soil microflora. In laboratory systems the amount of hyphae was lowest in replicates into which nitrogen had been added (BÄÄTH *et al.* 1981).

Combining these observations and what is known about nutritional biology of *C. sphagnetorum*, it seems plausible that the population responses were caused by changes in microflora in favour of bacteria. Direct harmful effect to animals may be involved at first in the case of ammonium nitrate, but the later development after this treatment remains unexplained (*cf.* field and laboratory experiments). When the acidity returned to the original level after urea treatment, there was no obstacle for fungi and enchytraeids to grow and reproduce. At this time (fourth year after fertilization) the enhanced primary production can be expected to have led to increasing amounts of decomposable litter. Fungal growth seems to be limited by available energy rather than nitrogen according to BÄÄTH *et al.* (1979). Food availability is regarded as the main factor for increase of enchytraeids after clear-cutting (HUHTA 1976, LUNDKVIST 1983). Experiments of BERG *et al.* (1980) indicate that *C. sphagnetorum* is capable of consuming pine needle litter. Higher N-content of litter may also be involved. ABRAHAMSEN & THOMPSON (1979) found a positive correlation between numbers of enchytraeids and total N content in organic matter. LATTER & HOWSON (1978) reported best growth of *C. sphagnetorum* on food with high N content.

Certainly the pH and nitrogen processes in soil are intercoupled in many ways. Increasing acidity leads to neutralization of ammonium-N which forms irreversibly complex compounds with soil organic matter (ROBERGE & KNOWLES 1966). Ammonium-N was found to increase after addition of ash in our study plots (UOMALA, unpublished).

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Synopsis: *Original scientific paper*

HUHTA, V., 1984. Response of *Cognettia sphagnetorum* (Enchytraeidae) to manipulation of pH and nutrient status in coniferous forest soil. *Pedobiologia* **27**, 245—260.

Effects of nitrogen and multiple non-nitrogen (ash + phosphorus) fertilizers on the populations of *Cognettia sphagnetorum* were studied in laboratory and field conditions. Laboratory tests were designed to separate the effects of pH from those of nutrients. Equal amounts of N were given as urea and ammonium nitrate, which result in different reactions in the soil pH. Ash + P was controlled by manipulating the pH to the same level with $\text{Ca}(\text{OH})_2$. All changes caused by non-nitrogen fertilization could be explained by pH alone. Both pH and nitrogen as a nutrient seem to be involved in the response caused by nitrogenous fertilizers.

Key words: *Cognettia sphagnetorum*, Enchytraeidae, nutrients, fertilization, pH, forest soil, raw humus.